

Vasoactive Intestinal Polypeptide

Distribution and possible role

in the upper respiratory and digestive regions

by Rolf Uddman

LUND 1980

VASOACTIVE INTESTINAL POLYPEPTIDE

Distribution and possible role in the upper respiratory and digestive regions

Akademisk avhandling som med vederbörligt tillstånd av Medicinska Fakulteten vid Universitetet i Lund för avläggande av medicine doktorsexamen kommer att offentligens försvaras i Universitetsklinikernas aula, Allmänna Sjukhuset i Malmö, onsdagen den 7 maj kl 09.15.

av

Rolf Uddman
Med lic, K1m

DOKUMENTDATABLAD enl SIS 61 41 21

Organisation LUND UNIVERSITY University of Lund Department of Otolaryngology Malmö General Hospital S-214 01 MALMÖ/Sweden		Document name DOCTORAL DISSERTATION	
Author(s) Rolf Uddman		Date of issue Maj 1980	
		CODEN: LUMEDW/ (MERM-1004) 1980	
		Sponsoring organisation	
Title and subtitle Vasoactive intestinal polypeptide - Distribution and possible role in the upper respiratory and digestive regions			
Abstract This study was performed in order to map the distribution and to evaluate some physiological aspects of <u>vasoactive intestinal peptide (VIP)</u> containing nerves in the upper respiratory and digestive regions. Immunohistochemical studies revealed a rich supply of VIP containing nerves in the <u>upper respiratory tract</u> , the <u>salivary glands</u> and the <u>oesophagus</u> of several mammals, including man. The nerve fibres were associated with smooth muscle, blood vessels, glandular acini and surface epithelium. The pterygopalatine ganglion harboured numerous VIP immunoreactive nerve cell bodies projecting to the <u>nasal mucosa</u> . In addition, VIP cell bodies were observed in the tracheal wall, the hilus region of the submandibular gland and in the myenteric and submucosal plexuses of the oesophagus. Pure porcine VIP evoked an atropine resistant dilatation of <u>resistance and capacitance vessels</u> in the feline nasal mucosa. Electrical stimulation of the Vidian nerve evoked a dilatation of nasal blood vessels concomitant with an increase of VIP in nasal venous blood. Electrical stimulation of the feline chorda lingual nerve caused dilatation of blood vessels in the submandibular gland concomitant with an increase of VIP in the venous effluent. Pure porcine VIP induced a dose-dependent relaxation of oesophageal smooth muscle in vitro.			
Key words Immunohistochemistry; neuropeptides; peptidergic nerves.		A4A5	
Classification system and/or index terms (if any)			
Supplementary bibliographical information		Language English	
ISSN and key title		ISBN	
Recipient's notes		Number of pages 96	
		Price	
		Security classification	

Distribution by (name and address) University of Lund, Department of Otolaryngology, Malmö General Hospital, S-214 01 MALMÖ/Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature _____

Date _____

VASOACTIVE INTESTINAL POLYPEPTIDE

Distribution and possible role in the upper respiratory and digestive regions

by

Rolf Uddman

From the Department of Otolaryngology
University of Lund
Malmö General Hospital, Malmö
and
the Department of Histology
University of Lund, Lund, Sweden

Lund 1980

CONTENTS

INTRODUCTION

Neuroanatomy of the upper respiratory and digestive regions
Design of the autonomic nervous system
VIP and other neuropeptides
Objectives of the study

METHODOLOGICAL CONSIDERATIONS

Immunohistochemistry
Antisera
Tissue processing
Immunohistochemical controls
Radioimmunoassay
Measurement of blood vessel tone in the nasal mucosa
Electrical stimulation of nerves
Collection of venous plasma effluents
In vitro studies on oesophageal smooth muscle

RESULTS

Occurrence and distribution of VIP nerve fibres in the upper respiratory tract
Occurrence and distribution of VIP nerve fibres in the salivary glands
Occurrence and distribution of VIP nerve fibres in the oesophagus
Effects of VIP on blood vessels in nasal mucosa
Effects of nervous stimulation on release of VIP into venous blood
Effects of VIP on oesophageal smooth muscle

DISCUSSION

Possible role of VIP containing nerves
Possible role of VIP
VIP in salivary glands
VIP in the upper respiratory tract
VIP in oesophagus
Clinical aspects



SUMMARY

ACKNOWLEDGEMENTS

REFERENCES

This thesis is based on the following papers which in the text will be referred to by their Roman numerals:

- I. Occurrence and distribution of VIP nerves in the nasal mucosa and tracheobronchial wall. *Acta Otolaryng (Stockh)* 86, 443-448, 1978. (Together with J. Alumets, O. Densert, R. Håkanson, and F. Sundler).
- II. Vasoactive intestinal polypeptide nerves in human upper respiratory tract. *ORL* 41, 221-226, 1979. (Together with F. Sundler).
- III. The origin of vasoactive intestinal polypeptide (VIP) nerves in the feline nasal mucosa. *Acta Otolaryng (Stockh)* 89, 152-156, 1980. (Together with L. Malm, and F. Sundler).
- IV. Innervation of the feline Eustachian tube. *Ann Otol* 88, 557-561, 1979. (Together with J. Alumets, O. Densert, M. Ekelund, R. Håkanson, I. Lorén, and F. Sundler).
- V. Neuronal VIP in salivary glands: Distribution and release. *Acta Physiol Scand.* (in press). (Together with J. Fahrenkrug, L. Malm, J. Alumets, R. Håkanson, and F. Sundler).
- VI. Peptidergic (VIP) innervation of the esophagus. *Gastroenterology* 75, 5-8, 1978. (Together with J. Alumets, L. Edvinsson, R. Håkanson, and F. Sundler).
- VII. A rich VIP nerve supply is characteristic of sphincters. *Nature* 280, 155-156, 1979. (Together with J. Alumets, J. Fahrenkrug, R. Håkanson, O. Schaffalitzky de Muckadell, and F. Sundler).
- VIII. Effects of vasoactive intestinal polypeptide (VIP) on resistance and capacitance vessels in the nasal mucosa. *Acta Otolaryng (Stockh)*. (in press). (Together with L. Malm, and F. Sundler).
- IX. VIP increases in venous nasal blood during stimulation of the Vidian nerve. *Acta Otolaryngol (Stockh)*. Submitted. (Together with L. Malm, and J. Fahrenkrug).

INTRODUCTION

Neuroanatomy of the upper respiratory and digestive regions

Exocrine secretion and vascular and non-vascular smooth muscle activity are important functions of the upper respiratory and digestive regions. These functions are regulated by the autonomic nervous system, classically divided into the sympathetic and parasympathetic systems. The postganglionic sympathetic fibres to the nasal mucosa, salivary glands and oesophagus originate in the cervical and thoracic ganglia of the sympathetic chain. Postganglionic parasympathetic nerve fibres have their cell bodies located close to, or within, the innervated organ. The postganglionic parasympathetic nerve fibres to the nasal mucosa have their cell bodies in the pterygopalatine and ciliary ganglia, both situated at a short distance from the target (Fig. 1). The pterygopalatine ganglion also contains cell bodies that give off axons to the Eustachian tube. Within the tracheal wall preganglionic parasympathetic nerve fibres terminate on cell bodies in small ganglia located in the smooth muscle in the posterior wall. The parotid gland receives its postganglionic parasympathetic nerve supply from the otic ganglion, situated at some distance from the gland, whereas the submandibular and sublingual glands receive their postganglionic parasympathetic fibres from cell bodies located in the hilus region and the glandular parenchyma. In the oesophagus nerve cell bodies are found in the myenteric plexus, located between the longitudinal and circular smooth muscle layer, and in the submucosal plexus, located between the circular smooth muscle and the muscularis mucosae. Both plexuses form a continuous chain of ganglia from the lower oesophagus down to the rectum. It is to be expected that this arrangement offers distinct advantages for local reflexes and for the modulation of the central impulse flow according to local needs.

The first detailed description of the ganglia innervating the upper respiratory tract and the salivary glands was published one hundred years ago (Retzius, 1880). The staining methods (methylene blue or silver impregnation techniques) used in early studies on the morphology of ganglia and the distribution of nerve fibres in the upper respiratory tract (Müller & Dahl, 1910), salivary glands (Huber, 1896) and oesophagus (Lawrentjew, 1929) did not allow distinction between different types of nerves. Not until the introduction of modern histochemical methods was it possible to describe the distribution and origin of the individual types of nerve fibres.

The Falck-Hillarp method demonstrates adrenergic nerve fibres (see Björklund et al., 1972). The method, and various modifications thereof (Lindvall & Björklund, 1974; Lorén et al., 1976), have been used extensively in studies

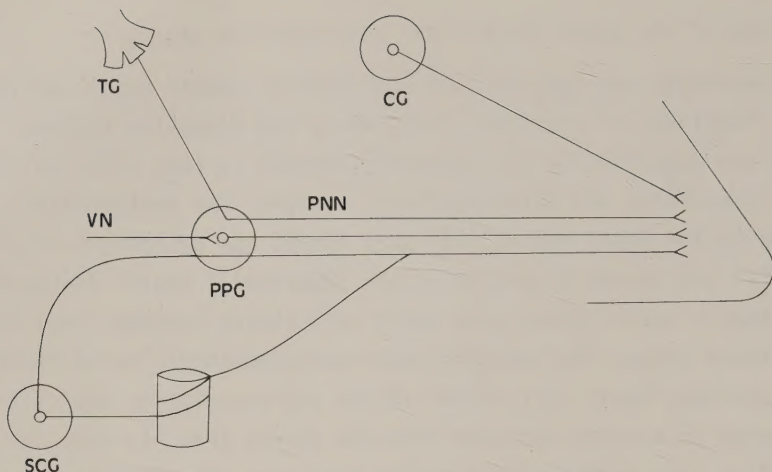


Fig. 1. Origin and course of autonomic nerves to the nasal mucosa. VN = Vidian nerve; PNN = Posterior nasal nerve; CG = Ciliary ganglion; TG = Trigeminal ganglion; PPG = Pterygopalatine ganglion; SCG = Superior cervical ganglion.

on the occurrence and distribution of adrenergic nerve endings in the upper respiratory tract (Dahlström & Fuxe, 1965; Dahlström et al., 1966; Nomura & Matsuura, 1972; Änggård & Densert, 1974; Grote et al., 1975), the salivary glands (Norberg & Olson, 1965; Garrett, 1967) and oesophagus (Baumgarten & Lange, 1969). In these organs the adrenergic fibres form networks around blood vessels and around the acini of seromucous glands. In addition, adrenergic nerves are associated with non-vascular smooth muscle and surface epithelia. In the oesophagus numerous adrenergic nerve fibres are present also in the myenteric and the submucosal plexuses.

The conventional histochemical method for staining "cholinergic" nerves demonstrates the enzyme acetylcholinesterase (AChE). The validity of the method is, however, questionable since certain adrenergic neurones, sensory neurones and various systems of aminergic nerve fibres in the brain have been found to contain this enzyme (Kyösola & Rechartt, 1974; Burnstock, 1978; Lehman & Fibiger, 1979). AChE-positive nerves and nerve cell bodies are numerous in the upper respiratory tract (Koelle & Koelle, 1959; Ishii, 1970; Mann, 1971; Nomura & Matsuura, 1972; Grote et al., 1975), the salivary glands (Garrett, 1966) and the oesophagus (Gerebtzoff & Bertrand, 1957). Generally, the AChE-positive nerve fibres occur around blood vessels, around the acini of seromucous glands, in smooth muscle and beneath surface epithelia.

With few exceptions the postganglionic sympathetic nerve fibres are noradrenergic. Postganglionic parasympathetic nerves have generally been thought of as cholinergic. The concept of a two-component autonomic nervous system fails, however, to take into account several ultramorphological and combined electrophysiological and pharmacological observations which all point to the existence of nerves which are neither adrenergic nor cholinergic (Burnstock, 1972).

Such observations have been made on a number of tissues including the nasal mucosa (Malm, 1973; Ånggård, 1974), the tracheal wall (Coburn & Tomita, 1973), the salivary glands (Bhoola et al., 1965) and the oesophagus (Gonella et al., 1979).

Among the pharmacological observations suggesting the existence of parasympathetic non-cholinergic mechanisms are e.g. the atropine resistant relaxation of the gut smooth muscle and the atropine resistant vasodilatation in the submandibular gland (Heidenhain, 1872) and in the nasal mucosa (Undritz, 1929). Further, already Langley (1898) observed that stimulation of vagal fibres induced a relaxation of the lower oesophageal sphincter. This relaxation was not affected by atropine, suggesting a non-cholinergic parasympathetic mechanism (Goyal & Rattan, 1975).

Ultrastructural observations indicate the existence of nerves which can be distinguished from the adrenergic and cholinergic ones on the basis of morphology and size of their predominating population of cytoplasmic vesicles (Fig. 2). Such nerves were first described in the gut by Baumgarten et al. (1970) and referred to as "p-type" (p for peptidergic) because of the morphological similarity of the vesicles with those in polypeptide-producing neurosecretory nerves in the neurohypophysis (Bargmann et al., 1967). Later studies have indicated the occurrence of several populations of "P-type" nerve fibres in the gut (Cook & Burnstock, 1976).

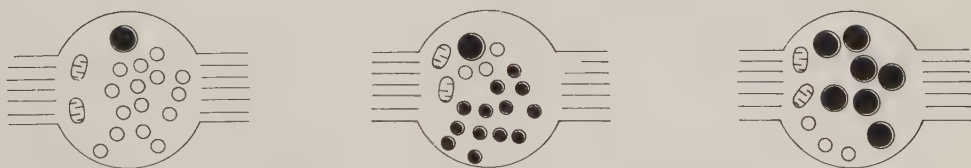


Fig. 2. Schematic drawing of the ultrastructure of different types of autonomic nerve endings. Left cholinergic, centre noradrenergic, right "p-type" (peptidergic).

VIP and other neuropeptides

In 1970 Said & Mutt isolated a vasodilatory polypeptide from the upper parts of porcine intestine. The peptide was named vasoactive intestinal polypeptide (VIP) although it was found to exert a wide range of biological actions besides vasodilatation. It was found to consist of 28 amino acid residues and its sequence was established by Mutt & Said (1974). VIP, secretin and glucagon belong to the same family of peptides and it is easy to understand why VIP was first thought of as a new gut hormone (Grossman, 1974). Subsequent immuno-histochemical studies revealed that VIP was localized in nerves rather than in endocrine cells. VIP containing nerves are numerous all along the gastrointestinal tract (Bryant et al., 1976; Larsson et al., 1976), the genito-urinary tract (Alm et al., 1977; Larsson et al., 1977) and the central nervous system (Larsson et al., 1976; Fuxe et al., 1977; Lorén et al., 1979).

Using immunohistochemical and immunochemical techniques several other peptides with putative neurotransmitter function were found to occur in neurones of both the peripheral and central nervous system. In the upper respiratory and digestive regions substance P immunoreactive nerve fibres have been observed (Hökfelt et al., 1975; Nilsson et al., 1975; Nilsson et al., 1977). Recently, a rich supply of enkephalin containing nerves was demonstrated in the oesophageal wall (Uddman et al., 1980). Several other peptides are claimed to occur in nerves in peripheral organs, notably the gut. Such peptides include somatostatin, angiotensin, neurotensin and gastrin/CCK (Schultzberg et al., 1978; Lundberg, 1979; Furness & Costa, 1980; Sundler et al., 1980). So far nothing is known about the distribution of these putative neuropeptides in the upper respiratory tract or salivary glands.

Objectives of the study

The present investigations had the following aims:

1. to study the occurrence, distribution and origin of VIP containing nerve fibres in the upper respiratory tract, salivary glands and oesophagus;
2. to study the release of VIP into nasal venous blood upon stimulation of the Vidian nerve and to study the vascular effects of VIP on the nasal mucosa;
3. to study the release of VIP into submandibular venous blood upon stimulation of the chorda lingual nerve;
4. to study the effect of VIP on oesophageal smooth muscle.

METHODOLOGICAL CONSIDERATIONS

Immunohistochemistry

The immunohistochemical procedures used were the indirect immunofluorescence method of Coons et al. (1955) and the peroxidase-antiperoxidase (PAP) procedure of Sternberger (1974). In the former method, specific antibodies (usually raised in rabbits) are allowed to react with the antigen in a tissue section. After rinsing the section is exposed to fluorescein-labelled sheep (or goat) antibodies raised against rabbit IgG. In this way the localization of the primary antigen-antibody complex is revealed by the fluorescent marker (Fig.3).

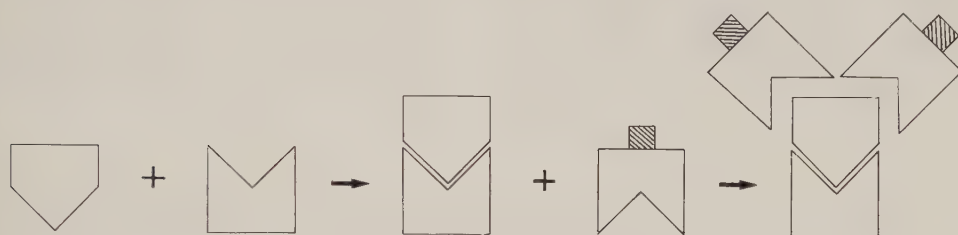


Fig. 3. Indirect immunofluorescence procedure. From left to right, rabbit antibody reacts with antigen in tissue section giving rise to antigen-antibody complex. After rinsing, a second antibody raised against rabbit IgG and labelled with fluorophor reacts with the first antibody.

The peroxidase-antiperoxidase procedure involves the use of a peroxidase-antiperoxidase complex, i.e. peroxidase coupled to a rabbit antibody against horseradish peroxidase. After the primary antibodies and the secondary unlabelled antirabbit IgG antibodies have been applied onto the tissue section, PAP complex is added to react with the second antibody. The main advantage of this method over the immunofluorescence technique is the greater sensitivity, allowing much higher (at least 10 fold) dilutions of the primary antibodies. For the light microscopical demonstration of peroxidase the conventional method of Karnovsky (involving diaminobenzidine as reagent) is used. The product is dark brown and has the additional advantage of becoming electron dense after treatment with osmium tetroxide, making the PAP method useful also for immunocytochemical studies at the electron microscopical level (Fig. 4).

Antisera

The validity of the immunohistochemical results depends on the quality (specificity and titer) of the antisera used. As a rule antisera against a peptide recognizes only a part of the amino acid sequence. In the present studies we have used VIP antisera (code No. 5603 and 98 P) raised against

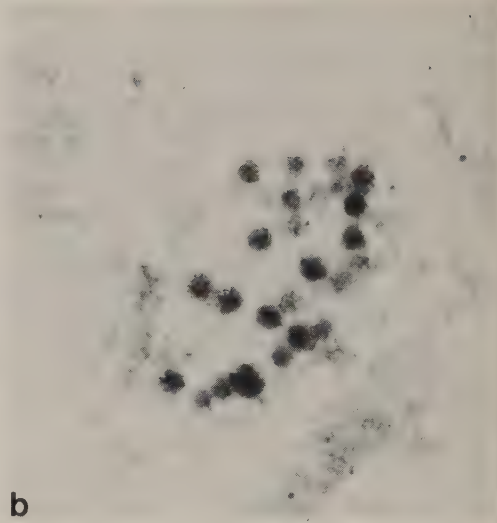
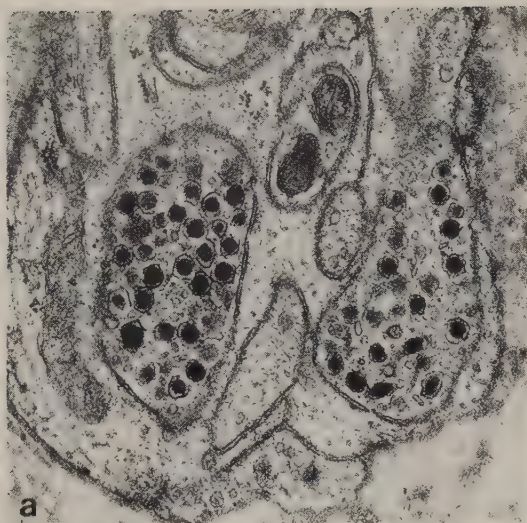


Fig. 4. Cat oesophagus. Fixation by formaldehyde-glutaraldehyde, postfixation by osmiumtetroxide. (a) "p-type" nerve profiles in smooth muscle layer, large dense cored vesicles predominate. (b) Electron immunocytochemical demonstration of VIP. Accumulation of reaction products over dense cored vesicles.

pure porcine VIP conjugated with bovine serum albumin. Antiserum 5603 is directed against the C-terminal portion of the VIP molecule (sequence 7-28). The antiserum has been characterized in detail elsewhere (Fahrenkrug & Schaffalitzky de Muckadell, 1978; Fahrenkrug, 1979). Antiserum 98 P (kind gift from Dr. S.I. Said, Dallas, Texas, USA) has been used in several immunohistochemical studies (e.g. Buffa et al., 1977; Sundler et al., 1979). It is not yet characterized with respect to region specificity.

Tissue processing

Freeze-dried specimens from the upper respiratory and digestive regions were fixed by exposure to diethylpyrocarbonate (DEPC) vapour (Pearse et al., 1974). The fixation properties of DEPC depends on its ability to cross-link carboxyl groups in peptides and proteins. Paraffin embedded sections were cut at 6 μ m, deparaffinized, hydrated and subjected to the indirect immunofluorescence method or to the PAP procedure for the demonstration of VIP.

Immunohistochemical controls

Unspecific staining is common in immunohistochemistry. For testing the specificity of the staining the antiserum was inactivated before its application to the sections. Antisera can be inactivated either by addition of antigen in excess or by elimination of the specific antibodies by e.g. affinity chroma-

tography (Vandesande, 1979). In the present studies, sections incubated with antiserum inactivated by the addition of pure porcine VIP (10-100 μ g per ml diluted antiserum) served as controls. The two VIP antisera used did not cross react with any other known hormonal or neuronal peptides such as secretin, gastric inhibitory peptide, pancreatic glucagon, gut-type glucagon, pancreatic polypeptide, substance P or somatostatin.

Cross reactivity of the VIP antisera with unknown peptides, containing the immunoreactive amino acid sequence, cannot be excluded. It would therefore be more appropriate to use expressions such as VIP-like immunoreactivity for the immunoreactive product. For brevity nerve fibres displaying VIP-like immunoreactivity are referred to as VIP fibres.

Radioimmunoassay

The concentration of VIP in plasma and tissue extracts was measured radio-immunochemically (Fahrenkrug & Schaffalitzky de Muckadell, 1977; Fahrenkrug, 1979). The sensitivity of this assay is such that it detects VIP in concentrations of 3.3 pmol/l plasma within a confidence limit of 95 %. For details on the radioimmunoassay of VIP, see Fahrenkrug & Schaffalitzky de Muckadell (1977).

Measurement of blood vessel tone in the nasal mucosa

To study the effects of VIP on nasal blood vessel tone the resistance and the capacitance functions of the nasal mucosa was measured. In order to reduce reflex activity on the nasal blood vessels the Vidian nerve and the cervical sympathetic nerve were cut prior to the experiments.

For the determination of changes in blood flow resistance (tone of resistance vessels) of the nasal mucosa a cannula was inserted in the vein in the pterygo-palatine foramen. As shown by Malm (1974) this vein seems to drain blood from the nasal mucosa only. The blood flow through the cannula was recorded by an optical flowmeter. The arterial blood pressure was continuously recorded via a catheter in the right femoral artery. The blood flow resistance was calculated as changes in regional vascular resistance (obtained by dividing the perfusion pressure with the regional venous blood flow). The perfusion pressure was measured as the difference between arterial blood pressure and venous blood pressure. Changes of blood flow resistance were expressed as per cent of the resting flow resistance.

Changes in the local blood content (tone of capacitance vessels) were recorded as pressure changes in a balloon placed in the nasal cavity on the same side

as the cannulated pterygopalatine vein. The balloon was given a distending pressure of 4-6 cm H₂O above atmospheric pressure. Nasal patency may be affected not only by changes in the capacitance vessels but also by trans-capillary fluid flow and by secretion from the seromucous glands. These mechanisms were considered to be of minor importance in the present studies (Mellander & Johansson, 1968; Malm, 1974).

Electrical stimulation of nerves

The nerves were stimulated by a Grass model S 44 stimulator with square wave pulses. The Vidian nerve was stimulated with pulses (2 ms, 20 Hz) of low intensity (1.5-3 V) or of high intensity (6-8 V). The chorda lingual nerve was stimulated with pulses well above the threshold values (6-8 V, 2 ms) in the following frequencies: 20, 10, 5, 2.5 and again 20 Hz. The cervical sympathetic nerve was also stimulated (4-6 V, 2 ms, 10 Hz).

Collection of venous plasma effluents

In chronically sympathectomized cats the Vidian nerve and the vein in the pterygopalatine foramen were exposed. The pterygopalatine vein was cannulated with a small polyethylene catheter. The Vidian nerve was electrically stimulated before and after administration of atropine and hexamethonium. During the stimulation blood from the pterygopalatine vein was collected and the plasma assayed for VIP.

In order to demonstrate a nervous release of VIP from the submandibular gland into venous plasma during electrical stimulation of the chorda lingual nerve, all tributaries to the external jugular vein, except a few veins from the submandibular gland, were ligated. The external jugular vein was then cannulated with a polyethylene catheter and a cannula was inserted in the submandibular duct. The peripheral end of the chorda lingual nerve was stimulated by pulses well above the threshold value. After the chorda lingual nerve stimulation the cut cervical sympathetic nerve was stimulated. During the stimulation periods blood from the external jugular vein was collected in ice-chilled tubes and plasma stored until radioimmunoassay of VIP.

In vitro studies on oesophageal smooth muscle

In many laboratory animals the oesophagus consists in its entire length of striated muscle, but for a narrow collar of smooth muscle at the oesophago-gastric junction. In man and in some other species such as the opossum and monkey, the lower third of the oesophagus consists of smooth muscle. Also in

the cat a segment of the lower oesophagus consists of smooth muscle. This animal was therefore chosen for a study of the effects of VIP on oesophageal smooth muscle.

The motor effects of VIP were analyzed in an in vitro system using circular smooth muscle from the cat oesophagus. Small strips were taken 5-7 cm or 1 cm above the oesophago-gastric junction. The muscle strips were mounted longitudinally and connected to a force-displacement transducer (Edvinsson & Owman, 1974). The strips were given a tension of 0.5-1 g and allowed to equilibrate for 1.5 h before starting the experiments. Isometric relaxation during VIP administration was then amplified and recorded.

R E S U L T S

Occurrence and distribution of VIP nerve fibres in the upper respiratory tract

VIP containing nerve fibres in the nasal mucosa and tracheobronchial wall were numerous in the cat and less numerous in the rabbit, guinea pig and man (Paper I,II). The over-all distribution of the VIP fibres was similar in all species. In the nasal mucosa a particularly rich supply of fine varicose VIP nerve fibres was noted in the maxilloturbinal area; other parts of the nasal mucosa contained a moderate supply of VIP fibres. In the epipharynx, mesopharynx and larynx few scattered VIP fibres were seen. Generally, VIP fibres were distributed beneath the surface epithelium, around small blood vessels and around the acini of seromucous glands. In the tracheobronchial wall a rich number of VIP fibres was in addition noted within and around bundles of smooth muscle. Clusters of VIP containing nerve cell bodies were observed in the posterior wall of the trachea of cat and man. This finding suggests that tracheobronchial VIP fibres emanate from local ganglia. In the nasal mucosa no VIP cell bodies were detected. The nasal VIP fibres could be shown to originate in the pterygopalatine ganglion (Paper III). This conclusion was based on the following observations: 1. The pterygopalatine ganglion harboured numerous VIP immunoreactive nerve cell bodies. 2. The posterior nasal nerve carried a large number of VIP containing fibres. 3. Extirpation of the pterygopalatine ganglion resulted in an almost complete loss of nasal VIP nerve fibres. 4. Extirpation of a segment of the Vidian nerve (decentralization of the ganglion) did not affect the number of VIP nerve fibres in the nasal mucosa. Axonal degeneration of nasal VIP nerve endings after extirpation of the pterygopalatine ganglion was slow, such fibres were absent not until after one week (Sundler et al., 1979). The distribution of adrenergic, AChE-positive and VIP containing nerve fibres was studied in the feline Eustachian tube (Paper IV). The different neuronal systems had roughly the same distribution

pattern. Generally, the nerve fibres were observed around small blood vessels, around acini of seromucous glands and beneath the epithelium.

Occurrence and distribution of VIP nerve fibres in the salivary glands

VIP nerve fibres were detected in salivary glands in all species studied; rat, cat and man (Paper V). The fibres were distributed around blood vessels and glandular acini. A dense accumulation of VIP fibres was noted around the salivary ducts where they were particularly numerous just beneath the epithelium. Also the main excretory ducts of the salivary glands were surrounded by numerous VIP fibres. There were great species differences both with regard to the density of the VIP containing nerve fibres and with regard to the concentration of VIP in the different salivary glands. Thus, VIP fibres in salivary glands were numerous in the cat, less so in the rat and man. A notable finding was the occurrence of clusters of VIP nerve cell bodies in the hilus region of cat submandibular gland. Radioimmunoassay revealed moderate amounts of VIP in the salivary glands of rat and man. In the cat the submandibular and sublingual glands contained high concentrations of VIP while the parotid and zygomatic glands contained smaller amounts. On the whole the values agreed with the immunohistochemical findings.

Occurrence and distribution of VIP nerve fibres in the oesophagus

VIP nerve fibres were observed in the oesophageal wall of all species studied; rat, cat and pig (Paper VI). They were numerous within the smooth muscle where they predominated in the circular muscle layer. In the entire length of the oesophagus VIP nerve fibres were seen in close association with small blood vessels and in the lamina muscularis mucosae. The myenteric and submucosal plexuses contained a dense network of immunoreactive fibres and also numerous VIP containing nerve cell bodies. The VIP nerve fibres became gradually more numerous in the lower parts of the oesophagus and a particularly dense accumulation was noted close to the oesophago-gastric junction (i.e. in the sphincter region). This distribution of VIP nerve fibres has been observed also in opossum, monkey and man (Uddman et al., unpublished observations). The dense accumulation of VIP nerve fibres in the oesophageal sphincter region stimulated a study of the VIP nerve supply to several other sphincters, recognized or anticipated (Paper VII). The pyloric sphincter and the sphincter of Oddi received a very dense VIP nerve supply. Also sphincters of the genito-urinary tract were rich in VIP nerve fibres. Immunochemical assay revealed higher VIP concentrations in the lower oesophageal sphincter than in adjacent non-sphincteric regions. The characteristic accumulation of VIP nerve fibres in smooth

muscle sphincters strongly suggests that VIP is involved in the regulation of sphincter tonus.

Effects of VIP on blood vessels in nasal mucosa

When given via the lingual artery VIP caused a dose-dependent reduction in tone of the resistance and capacitance vessels (Paper VIII). At high doses the systemic arterial blood pressure was lowered, indicating systemic effects of VIP. The vasodilatory effect of VIP was not affected by atropine.

Effects of nervous stimulation on release of VIP into venous blood

In sympathectomized cats (extirpation of the superior cervical ganglion) electrical stimulation of the Vidian nerve evoked a vasodilatation concomitant with a marked increase in the concentration of VIP in the venous effluent (Paper IX). Atropine did not prevent the vasodilatation nor the VIP release. The ganglionic blocker hexamethonium prevented the VIP increase and reduced the vasodilatation following nerve stimulation.

Electrical stimulation of the chorda lingual nerve evoked a frequency dependent increase in venous blood flow and saliva secretion (Paper V). Also the plasma concentration of VIP increased with increasing stimulation frequencies. Stimulation of the cervical sympathetic nerve evoked a decreased blood flow while the concentration of VIP in venous plasma effluent was unaffected. VIP could not be detected in saliva.

Effects of VIP on oesophageal smooth muscle

Exogenous VIP reduced the passive tension of oesophageal smooth muscle strips (Paper VI). In order to induce a long-lasting tonic contraction carbamylcholine was applied. Cumulative application of VIP resulted in a dose-dependent relaxation in the strips. Strips from the oesophago-gastric junction differed from those taken 5-7 cm above the junction by being more sensitive to VIP.

DISCUSSION

Possible role of VIP containing nerves

In the present investigations numerous VIP containing nerves were demonstrated in the upper respiratory tract, the salivary glands and the oesophagus. They were associated with blood vessels, smooth muscle, seromucous glands, ductal epithelia and surface epithelia. It is possible, even likely, that these structures represent targets for the VIP nerve fibres. The distribution of the VIP fibres conforms with many of the known biological actions of the peptide: dilatation of blood vessels, relaxation of smooth muscle and augmentation of secretion from exocrine glands.

The discovery that peptidergic nerves form a separate component of the autonomic nervous system, distinct from the adrenergic and the cholinergic ones (Fig. 5), have called for a reappraisal of the way the autonomic nervous system is organized. The physiological role of neuropeptides, such as substance P, VIP, somatostatin and enkephalin, is still unknown. It is not inconceivable that they act as neurotransmitters.

Possible role of VIP

The criteria for accepting a compound as a neurotransmitter have been discussed by Werman (1966) and more recently by Orrego (1979). According to these authors the primary criteria are:

1. Storage in neuro-vesicles
2. Induced release
3. Identity of action

A transmitter candidate should be stored in specific cytoplasmic organelles, vesicles, within the nerve terminal. Nerve stimulation should result in a release of the transmitter candidate in proportion to the magnitude of the stimulation. A transmitter candidate should be able to reproduce the post-synaptic effects of the natural transmitter. On the whole, available evidence favour the view that VIP is a neurotransmitter. VIP is stored in vesicles in "p-type" nerve terminals (Giachetti et al., 1977; Larsson et al., 1979; Sundler et al., 1980)(Fig. 4). VIP is released by a calcium-dependent potassium evoked mechanism (Emson et al., 1978). Nervous stimulation releases VIP into venous plasma effluent (Fahrenkrug et al., 1978; Paper V, Paper IX). The vascular effects of exogenous VIP mimics that of nerve stimulation (see for instance Paper VIII).

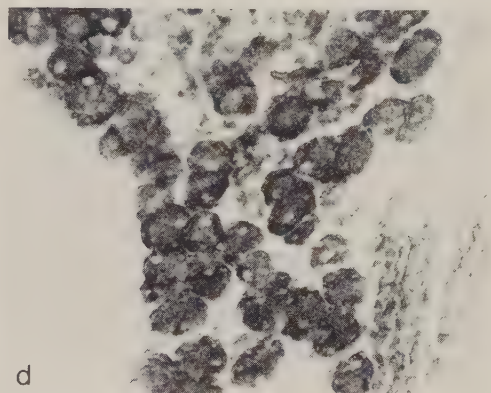
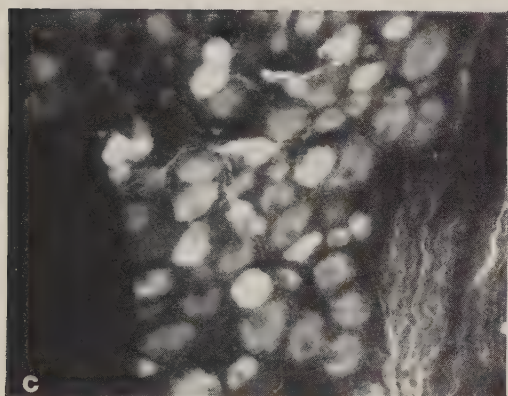
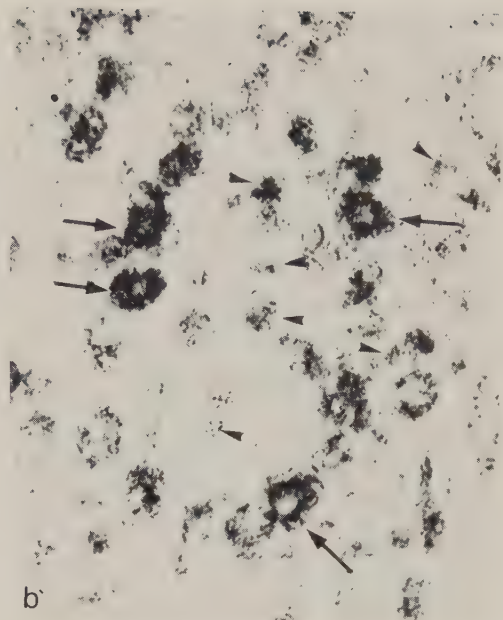
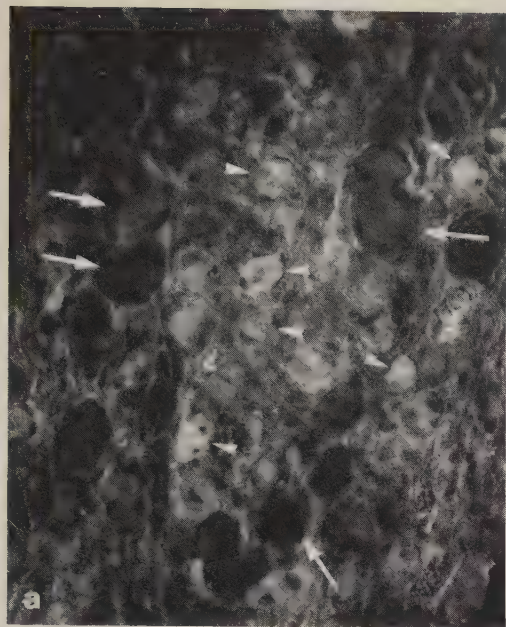


Fig. 5. Pterygopalatine ganglion. Cryostat section processed first for VIP immunofluorescence (a) and then for the demonstration of AChE (b). The cell bodies displaying intense VIP immunofluorescence contain no or very low AChE-activity (arrow heads) while those containing high AChE-activity are devoid of immunoreactive VIP (arrows). After local application of colchicine (c,d) for 6 h virtually all nerve cell bodies display VIP immunofluorescence and are AChE-positive. The result emphasizes the fact that AChE is a poor marker for cholinergic nerves.

VIP in salivary glands

Chorda lingual nerve stimulation causes vasodilatation and saliva secretion in the submandibular gland. Vasodilatation is atropine resistant, while secretion is not (Heidenhain, 1872). It was therefore a long held view that the chorda lingual nerve carried separate vasodilator and secretory fibres. This view was challenged by Barcroft & Piper (1912) who suggested that the vasodilator response was secondary to the local metabolic activity. Subsequently it was proposed that the vasodilatation was due to the release of the enzyme kallikrein. This enzyme was thought to pass from the exocrine cells into the extracellular space to act on plasma proteins to form a potent vasodilator agent, bradykinin (Ungar & Parrot, 1936; Hilton & Lewis, 1955). Kallikrein was also demonstrated in nasal secretion (Eccles & Wilson, 1973). On the other hand, Bhoola et al. (1965) noted that nervous stimulation elicited a vasodilator response in the submandibular gland also when it was desensitized to bradykinin. Thus, the mechanism behind the atropine resistant vasodilatation has remained a mystery. Shimizu & Taira (1979) tested the response of dog submandibular gland to graded infusions of VIP. They observed that VIP evoked a dose-dependent increase in the local blood flow. The vasodilatation could not be blocked by atropine and they suggested that the atropine resistant, neurally mediated vasodilatation in the gland could be caused by VIP. In fact the following arguments favour this view: 1. VIP containing nerves are numerous around blood vessels in the salivary glands. 2. Electrical stimulation of the chorda lingual nerve causes release of VIP into the venous effluent. 3. The effect of VIP on salivary blood flow (Shimizu & Taira, 1979) mimics chorda lingual nerve stimulation.

VIP in the upper respiratory tract

In the upper respiratory tract VIP containing nerve fibres occurred around blood vessels, around acini of seromucous glands, close to surface epithelium, and in the tracheal smooth muscle. In the nasal mucosa VIP caused a dose-dependent dilatation of both resistance and capacitance vessels. Also this dilatation was found to be atropine resistant. Electrical stimulation of the Vidian nerve evoked a vasodilatation concomitant with an atropine resistant increase of VIP in venous plasma effluent from the nasal mucosa. These findings suggest that also here VIP is a mediator of the neurally mediated vasodilatory response.

VIP stimulates secretion from certain exocrine glands (Domschke et al., 1977) and has potent relaxatory effects on tracheal smooth muscle (Said et al., 1974). We therefore suggest that VIP has the following actions in the upper respira-

tory tract: dilatation of blood vessels, regulation of secretion from sero-mucous glands and relaxation of tracheal smooth muscle.

VIP in oesophagus

The gut harbours several neuropeptides (Schultzberg et al., 1979; Sundler et al., 1980). While immunohistochemistry has provided detailed information of their distribution, information of their function is very limited. VIP nerves have a widespread distribution in all parts of the gastrointestinal tract. A notable finding is the dense accumulation of VIP nerve fibres around sphincters and we have therefore proposed that evaluation of the density of VIP nerve fibres may help in defining a sphincter. The fact that VIP has a potent relaxatory effect on oesophageal smooth muscle suggests that VIP participates in the neurally mediated relaxation of the lower oesophageal sphincter.

Clinical aspects

The occurrence of peptide containing nerves in the upper respiratory and digestive regions may have clinical implications. For instance the symptoms of perennial rhinitis have been assumed to reflect stimulation of parasympathetic nerves (Malcomson, 1959). Sectioning of the Vidian nerve has been tried in attempts to reduce the parasympathetic influence on the nasal mucosa (Golding-Wood, 1973). Recent studies indicate, however, that recurrence of the symptoms are common (Krant et al., 1979). VIP nerves are numerous around glandular acini and blood vessels in the nasal mucosa. VIP containing nerve cell bodies in the pterygopalatine ganglion are not affected by extirpation of the Vidian nerve. It is therefore not inconceivable that the VIP containing neurones are involved in the pathogenesis of this disease. Achalasia is a disorder characterized by a defective peristalsis in the body of the oesophagus and a failure of the oesophagus to relax properly upon swallowing. Preliminary data (Uddman et al., to be published) indicate that patients with achalasia have a reduced VIP concentration in the lower oesophageal sphincter region and much fewer VIP immunoreactive nerve fibres in the oesophageal smooth muscle as compared to controls. Conceivably a local loss of VIP fibres may be of significance in the pathogenesis of this disorder.

S U M M A R Y

1. Nerve fibres displaying vasoactive intestinal polypeptide (VIP) immunoreactivity were detected in the upper respiratory tract, salivary glands and oesophagus of several mammals, including man. VIP immunoreactive nerve cell bodies were encountered in the pterygopalatine ganglion, the tracheal wall, the hilus region of the submandibular gland and the myenteric and submucosal plexuses of the oesophagus. The nerve fibres were associated with smooth muscle, blood vessels, glandular acini, ductal epithelia and surface epithelia.
2. Pure porcine VIP given intraarterially close to the nasal mucosa evoked an atropine resistant dilatation of both resistance and capacitance vessels mimicking the effects of electrical stimulation of the Vidian nerve. In chronically sympathectomized cats electrical stimulation of the Vidian nerve evoked an atropine resistant vasodilatation as well as an increase of VIP in nasal venous effluent.
3. Electrical stimulation of the chorda lingual nerve stimulated salivary secretion and blood flow from the submandibular gland and raised the concentration of VIP in the venous plasma effluent.
4. Pure porcine VIP induced a dose-dependent relaxation of oesophageal smooth muscle in vitro. The sphincter was more sensitive to VIP than the oesophageal body.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to:

Professor Nils Emmelin for the use of the facilities of the Physiological Department, University of Lund, Lund;

Professor Bengt Falck for the use of the facilities of the Histological Department, University of Lund, Lund;

Docent Nils Gunnar Toremalm, Department of Otolaryngology, Malmö General Hospital, for taking an interest in my research work and for his support.

I have received invaluable technical assistance from Eva Engelbert, Kristine Fogelström and Marianne Maxe-Colschen.

I am also indebted to Bodil Nilsson for skillful typing of the various manuscripts.

This work was supported by grants from the Medical Faculty, University of Lund, from the Swedish Medical Research Council (Project No. 04X-4499) and from Albert Pahlsson's Foundation.

REFERENCES

- Alm, P., Alumets, J., Håkanson, R. & Sundler, F. 1977. Peptidergic (vasoactive intestinal peptide) nerves in the genito-urinary tract. *Neuroscience* 2, 751-754.
- Ånggård, A. 1974. The effects of parasympathetic nerve stimulation on the microcirculation and secretion in the nasal mucosa of the cat. *Acta Otolaryngol* (Stockh) 78, 98-105.
- Ånggård, A. & Densert, O. 1974. Adrenergic innervation of the nasal mucosa in cat. *Acta Otolaryngol* (Stockh) 78, 232-241.
- Barcroft, J. & Piper, H. 1912. The gaseous metabolism of the submaxillary gland with reference especially to the effect of adrenalin and the time relation of the stimulus to the oxidation process. *J Physiol* 44, 359-373.
- Bargmann, W., Lindner, E. & Andres, K.H. 1967. Über Synapsen an endokrinen Epithelzellen und die Definition sekretorischer Neurone. *Z Zellforsch* 77, 282-298.
- Baumgarten, H.G. & Lange, W. 1969. Adrenergic innervation of the oesophagus in the cat (*Felis domestica*) and rhesus monkey (*Macacus rhesus*). *Z Zellforsch* 95, 529-545.
- Baumgarten, H.G., Holstein, A.-F. & Owman, Ch. 1970. Auerbach's plexus of mammals and man: electron microscopic identification of three different types of neuronal processes in myenteric ganglia of the large intestine from rhesus monkeys, guinea-pigs and man. *Z Zellforsch* 106, 376-397.
- Bhoola, K.D., Morley, J., Schachter, M. & Smaje, L.H. 1965. Vasodilatation in the submaxillary gland of the cat. *J Physiol* 179, 172-184.
- Björklund, A., Falck, B. & Owman, Ch. 1972. Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In *Methods of Investigative and Diagnostic Endocrinology*. Vol 1: *The Thyroid and Biogenic Amines* (eds. J.E. Rall & I.J. Kopin), pp. 318-368. North-Holland Publ Co, Amsterdam.
- Bryant, M.G., Bloom, S.R., Polak, J.M., Albuquerque, R.H., Modlin, I. & Pearse, A.G.E. 1976. Possible dual role for vasoactive intestinal peptide as gastrointestinal hormone and neurotransmitter substance. *Lancet* 1, 991 - 993.
- Buffa, R., Capella, C., Solcia, E., Frigerio, B. & Said, S.I. 1977. Vasoactive intestinal peptide (VIP) cells in the pancreas and gastro-intestinal mucosa. *Histochemistry* 50, 217-227.
- Burnstock, G. 1972. Purinergic nerves. *Pharmacol Rev* 24, 509-580.
- 1978. Do some sympathetic neurones synthesize and release both noradrenaline and acetylcholine? *Prog Neurobiol* 11, 205-222.

- Coburn, R.F. & Tomita, T. 1973. Evidence for nonadrenergic inhibitory nerves in the guinea pig trachealis muscle. *Am J Physiol* 224, 1072-1080.
- Cook, R.D. & Burnstock, G. 1976. The ultrastructure of Auerbach's plexus in the guinea-pig. 1. Neuronal elements. *J Neurocytol* 5, 171-194.
- Coons, A.H., Leduc, E.H. & Conolly, J.M. 1955. Studies on antibody production. 1. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. *J Exp Med* 102, 49-60.
- Dahlström, A. & Fuxe, K. 1965. The adrenergic innervation of the nasal mucosa of certain mammals. *Acta Otolaryngol* (Stockh) 59, 65-72.
- Dahlström, A., Fuxe, K., Hökfelt, T. & Norberg, K.-A. 1966. Adrenergic innervation of the bronchial muscle of the cat. *Acta Physiol Scand* 66, 507-508.
- Domschke, S., Domschke, W., Rösch, W., Konturek, S.J., Sprügel, W., Mitznegg, P., Wünsch, E. & Demling, L. 1977. Vasoactive intestinal peptide: A secretin-like partial agonist for pancreatic secretion in man. *Gastroenterology* 73, 478-480.
- Eccles, R. & Wilson, H. 1973. A kallikrein-like substance in cat nasal secretion. *Br J Pharmacol* 49, 712-714.
- Edvinsson, L. & Owman, Ch. 1974. Pharmacological characterization of adrenergic alpha and beta receptors mediating vasomotor response of cerebral arteries in vitro. *Circ Res* 35, 835-849.
- Emson, P.C., Fahrenkrug, J., Schaffalitzky de Muckadell, O., Jessell, T.M. & Iversen, L.L. 1978. Vasoactive intestinal polypeptide (VIP): vesicular localization and potassium evoked release from rat hypothalamus. *Brain Res* 143, 174-178.
- Fahrenkrug, J. & Schaffalitzky de Muckadell, O. 1977. Radioimmunoassay of vasoactive intestinal polypeptide (VIP) in plasma. *J Lab Clin Med* 89, 1379-1388.
- 1978. Distribution of vasoactive intestinal polypeptide (VIP) in the porcine central nervous system. *J Neurochem* 31, 1445-1451.
- Fahrenkrug, J. 1979. Vasoactive intestinal polypeptide: measurement, distribution and putative neurotransmitter function. *Digestion* 19, 149-169.
- Furness, J.B. & Costa, M. 1980. Types of nerves in the enteric nervous system. *Neuroscience* 5, 1-20.
- Fuxe, K., Hökfelt, T., Said, S.I. & Mutt, V. 1977. Vasoactive intestinal polypeptide and the nervous system: Immunohistochemical evidence for localization in central and peripheral neurons, particularly intracortical neurons of the cerebral cortex. *Neurosci Lett* 5, 241-246.
- Garrett, J.R. 1966. The innervation of salivary glands. *J R Micro Soc* 85, 135-148.
- 1967. The innervation of normal human submandibular and parotid salivary glands. *Arch Oral Biol* 12, 1417-1436.

- Gerebtzoff, M.-A. & Bertrand, J. 1957. Gradients d'activité cholinestérasique dans la muqueuse du tube digestif. *Ann. Histochem* 2, 149-162.
- Giachetti, A., Said, S.I., Reynolds, R.C. & Koniges, F.C. 1977. Vasoactive intestinal polypeptide in brain: Localization in and release from isolated nerve terminals. *Proc Natl Acad Sci USA* 74, 3424-3428.
- Golding-Wood, P.H. 1973. Vidian neurectomy: its results and complications. *Laryngoscope* 83, 1673-1683.
- Gonella, J., Niel, J.P. & Roman, C. 1979. Sympathetic control of lower oesophageal sphincter motility in the cat. *J Physiol* 287, 177-190.
- Goyal, R. & Rattan, S. 1975. Nature of the vagal inhibitory innervation to the lower esophageal sphincter. *J Clin Invest* 55, 1119-1126.
- Grossman, M.I. 1974. Candidate hormones of the gut. *Gastroenterology* 67, 730-755.
- Grote, J.J., Kuipers, W. & Huygen, P.L.M. 1975. Selective denervation of the autonomic nerve supply of the nasal mucosa. *Acta Otolaryngol* (Stockh) 79, 124-132.
- Heidenhain, R. 1872. Ueber die Wirkung einiger Gifte auf die Nerven der glandula submaxillaris. *Arch Ges Physiol* 5, 309-318.
- Hilton, S.M. & Lewis, G.P. 1955. The mechanism of the functional hyperaemia in the submandibular salivary gland. *J Physiol* 129, 253-271.
- Hökfelt, T., Kellerth, J.O., Nilsson, G. & Pernow, B. 1975. Substance P: Localization in the central nervous system and in some primary sensory neurons. *Science* 190, 889-890.
- Huber, C. 1896. Observations on the innervation of the sublingual and submaxillary glands. *J Exp Med* 1, 281-295.
- Ishii, T. 1970. The cholinergic innervation of the human nasal mucosa. *Pract Oto-rhino-laryngol* 32, 153-158.
- Koelle, W.A. & Koelle, G.B. 1959. The localization of external or functional acetylcholinesterase at the synapses of autonomic ganglia. *J Pharmacol Exp Ther* 126, 1-8.
- Krant, J.N., Wildervanck de Blécourt, P., Dieges, P.H. & de Heer, L.J. 1979. Long-term results of Vidian neurectomy. *Rhinology* 17, 231-235.
- Kyösola, K. & Rechardt, L. 1974. The anatomy and innervation of the sphincter of Oddi in the dog and cat. *Am J Anat* 140, 497-522.
- Langley, J.N. 1898. On inhibitory fibres in the vagus for the end of the oesophagus and the stomach. *J Physiol* 23, 407-414.
- Larsson, L.-I., Fahrenkrug, J., Schaffalitzky de Muckadell, O., Sundler, F., Håkanson, R. & Rehfeld, J.F. 1976. Localization of vasoactive intestinal polypeptide (VIP) to central and peripheral neurons. *Proc Natl Acad Sci USA* 73, 3197-3200.

- Larsson, L.-I., Fahrenkrug, J. & Schaffalitzky de Muckadell, O. 1977. Vasoactive intestinal polypeptide occurs in nerves of the female genito-urinary tract. *Science* 197, 1374-1375.
- Larsson, L.-I., Polak, J.M., Buffa, R., Sundler, F. & Solcia, E. 1979. On the immunocytochemical localization of the vasoactive intestinal polypeptide. *J Histochem Cytochem* 27, 936-938.
- Lawrentjew, B.J. 1929. Über den Aufbau der Ganglien der Speiseröhre nebst einigen Bemerkungen über das Vorkommen und die Verteilung zweier Arten von Nervenzellen in dem autonomen Nervensystem. *Z Mikrosk Anat Forsch* 18, 233-262.
- Lehmann, J. & Fibiger, H.C. 1979. Acetylcholinesterase and the cholinergic neuron. *Life Sci* 25, 1939-1947.
- Lindvall, O. & Björklund, A. 1974. The glyoxylic acid fluorescence histochemical method: a detailed account of the methodology for the visualization of central catecholamine neurons. *Histochemistry* 39, 97-127.
- Lorén, I., Björklund, A., Falck, B. & Lindvall, O. 1976. An improved histo-fluorescence procedure for freeze-dried paraffin-embedded tissue based on combined formaldehyde-glyoxylic acid perfusion with high magnesium content and acid pH. *Histochemistry* 49, 177-192.
- Lorén, I., Emson, P.C., Fahrenkrug, J., Björklund, A., Alumets, J., Håkanson, R. & Sundler, F. 1979. Distribution of vasoactive intestinal polypeptide in the rat and mouse brain. *Neuroscience* 4, 1953-1976.
- Lundberg, J.M. 1979. Enkephalin, substance P, VIP, somatostatin, gastrin/CCK and neurotensin in peripheral neurons. *Acta Physiol Scand Suppl.* 473, 14.
- Malcomson, G. 1959. The vasomotor activities of the nasal mucous membrane. *J Laryngol Otol* 73, 73-98.
- Malm, L. 1973. Vasodilatation in the nasal mucosa of the cat and the effects of parasympatholytic and β -adrenergic blocking agents. *Acta Otolaryngol* (Stockh) 76, 277-282.
- 1974. Responses of resistance and capacitance vessels in feline nasal mucosa to vasoactive agents. *Acta Otolaryngol* (Stockh) 78, 90-97.
- Mann, S. 1971. The innervation of mammalian bronchial smooth muscle: the localization of catecholamines and cholinesterases. *Histochemical J* 3, 319-331.
- Mellander, S. & Johansson, B. 1968. Control of resistance, exchange and capacitance functions in peripheral circulation. *Pharmacol Rev* 20, 117-196.
- Müller, L.R. & Dahl, W. 1910. Die Beteiligung des sympathischen Nervensystems an der Kopfinnervation. *Dtsch Arch Klin Med* 99, 48-107.

- Mutt, V. & Said, S.I. 1974. Structure of the porcine vasoactive intestinal octacosapeptide. The amino acid sequence. Use of kallikrein in its determination. *Eur J Biochem* 42, 581-589.
- Nilsson, G., Larsson, L.-I., Håkanson, R., Brodin, E., Pernow, B. & Sundler, F. 1975. Localization of substance P-like immunoreactivity in mouse gut. *Histochemistry* 43, 97-99.
- Nilsson, G., Dahlberg, K., Brodin, E., Sundler, F. & Strandberg, K. 1977. Distribution and constrictor effect of substance P in guinea-pig tracheo-bronchial tissue. In *Substance P* (eds. U.S. von Euler & B. Pernow), pp. 75-81. Raven Press, N.Y.
- Nomura, Y. & Matsuura, T. 1972. Distribution and clinical significance of the autonomic nervous system in the human nasal mucosa. *Acta Otolaryngol* (Stockh) 73, 493-501.
- Norberg, K.-A. & Olson, L. 1965. Adrenergic innervation of the salivary glands in the rat. *Z Zellforsch* 68, 183-189.
- Orrego, F. 1979. Criteria for the identification of central neurotransmitters, and their application to studies with some nerve tissue preparations in vitro. *Neuroscience* 4, 1037-1057.
- Pearse, A.G.E., Polak, J.M., Adams, C. & Kendall, P.A. 1974. Diethylpyrocarbonate, a vapour-phase fixative for immunofluorescence studies on polypeptide hormones. *Histochem J* 6, 347-352.
- Retzius, G. 1880. Untersuchungen über die Nervenzellen der cerebrospinalen Ganglien und der übrigen peripherischen Kopfganglien. *Arch Anat Physiol* 369-402.
- Said, S.I. & Mutt, V. 1970. Polypeptide with broad biological activity: Isolation from small intestine. *Science* 169, 1217-1218.
- Said, S.I., Kitamura, S., Yoshida, T., Preskitt, J. & Holden, L.D. 1974. Humoral control of airways. *Ann N Y Acad Sci* 221, 103-114.
- Schultzberg, M., Dreyfus, C.H., Gershon, M.D., Hökfelt, T., Elde, R.P., Nilsson, G., Said, S.I. & Goldstein, M. 1978. VIP-, enkephalin, substance P-, and somatostatin-like immunoreactivity in neurons intrinsic to the intestine: immunohistochemical evidence from organotype tissue cultures. *Brain Res* 155, 239-248.
- Shimizu, T. & Taira, N. 1979. Assessment of the effects of vasoactive intestinal peptide (VIP) on blood flow through and salivation of the dog salivary gland in comparison with those of secretin, glucagon and acetylcholine. *Br J Pharmacol* 65, 683-687.
- Sternberger, L.A. 1974. *Immunocytochemistry*. Prentice-Hall Inc., Englewood Cliffs, N.J.

- Sundler, F., Alumets, J., Fahrenkrug, J., Håkanson, R. & Schaffalitzky de Muckadell, O. 1979. Cellular localization and ontogeny of immunoreactive vasoactive intestinal polypeptide (VIP) in the chicken gut. *Cell Tiss Res* 196, 193-201.
- Sundler, F., Malm, L. & Uddman, R. 1979. Slow axonal degeneration of peripheral VIP nerves. *Neurosci Lett*, Suppl. 3, 198.
- Sundler, F., Håkanson, R. & Leander, S. 1980. Peptidergic nervous systems in the gut. *Clin Gastroenterol.* (in press).
- Uddman, R., Alumets, J., Håkanson, R., Sundler, F. & Walles, B. 1980. Peptidergic (Enkephalin) innervation of the mammalian esophagus. *Gastroenterology.* (in press).
- Undritz, W. 1929. Über vasomotorische Reflexe der Nase. *Z Hals-Nasen-Ohrenheilkd* 25, 157-186.
- Ungar, G. & Parrot, J-L. 1936. Sur la présence de la callicréine dans la salive, et la possibilité de son intervention dans la transmission chimique de l'influx nerveux. *C R Acad Sci* 122, 1052-1055.
- Vandesande, F. 1979. A critical review of immunocytochemical methods for light microscopy. *J Neurosci Methods* 1, 3-23.
- Werman, R. 1966. Criteria for identification of a central nervous system transmitter. *Comp Biochem Physiol* 18, 745-766.

